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CLAIMS

[Claim(s)]
[Claim 1]A monoclonal antibody to which it is a monoclonal antibody which recognizes an ovomucoid, and a thermal denaturation ovomucoid does not react although it reacts to a native ovomucoid, A monoclonal antibody to which a native ovomucoid does not react although it reacts to a thermal denaturation ovomucoid, a monoclonal antibody reacted to a native ovomucoid and a thermal denaturation ovomucoid, and a hybridoma group which produces these.
[Claim 2]How to manufacture a monoclonal antibody group of claim 1 by uniting a mouse, a rat spleen cell and a mouse, or a rat myeloma cell which can produce an antibody to un-denaturalizing and a thermal denaturation ovomucoid, considering it as a hybridoma, and cultivating this hybridoma.
[Claim 3]It is a monoclonal antibody which recognizes an ovomucoid manufactured using a method of claim 2, (a) A monoclonal antibody to which a thermal denaturation ovomucoid does not react although it reacts to a native ovomucoid, (b) A monoclonal antibody and (c) to which a native ovomucoid does not react although it reacts to a thermal denaturation ovomucoid An immunological determination method of an ovomucoid using a monoclonal antibody reacted to a native ovomucoid and a thermal denaturation ovomucoid.
[Claim 4]It is a monoclonal antibody which recognizes an ovomucoid manufactured using a method of claim 2, (a) A monoclonal antibody to which a thermal denaturation ovomucoid does not react although it reacts to a native ovomucoid, (b) A monoclonal antibody and (c) to which a native ovomucoid does not react although it reacts to a thermal denaturation ovomucoid A manufacturing method of egg white protein from which an ovomucoid using a monoclonal antibody reacted to a native ovomucoid and a thermal denaturation ovomucoid was removed.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates the thermal denaturation state of a hen's egg white ovomucoid to an identifiable monoclonal antibody group, the hybridoma groups which produce them, and those manufacturing methods and uses.
[0002]
[Description of the Prior Art]It is said that one person has one person in three persons, and has a certain allergosis in the family now. A food allergy poses a much more serious problem from that foodstuffs show the symptoms of maintenance and growth of a life by high frequency focusing on the infants who influence greatly, and the shift to bronchial asthma etc. taking place so that it may be said with an allergy march that sensitization is once materialized especially. Although an egg, cow's milk, a soybean, rice, wheat, etc. are mentioned as main foodstuffs containing allergen, frequency of allergy to eggs is the highest at infants (Ministry-of-Health-and-Welfare food allergy measure examination committee Heisei 11 yearly progress report document). Ovalbumin, ovotransferrin, and an ovomucoid are mentioned as egg allergen. The ovomucoid is especially considered to be the most powerful allergen from the thermal stability or the tolerance over a digestive enzyme. The foundations of allergy treatments are not taking in allergen. Therefore, they have been identification of allergen, and a technical problem with important exact fixed quantity and its removal.
[0003]Identification of an ovomucoid, About a fixed quantity. it being alike by **** and a polyclonal antibody. A used method (Int. Archs. Allergy.) The method (J. Nutr. Sci. Vitaminol. 45, 491-500, 1999) using appl. Immun., 75, 8-15, 1984, or a monoclonal antibody is publicly known. Each of these considers that an ovomucoid is a native single state. However, what we eat actually is the protein (ovomucoid) which was heat-treated in almost all cases, if allergenic nature is made an issue of at all, the method in consideration of the existence state of the allergen in food must be taken, but such a method has not been developed yet.
[0004]
[Problem(s) to be Solved by the Invention]This invention makes it a technical problem to establish the method of producing the monoclonal antibody which a native ovomucoid, a thermal denaturation ovomucoid, and both recognize, identifying a heat denaturation state using these, and quantifying an ovomucoid.
[0005]

[Means for Solving the Problem]By the deovomucoid method aiming at preparation of reduction in allergen, or an allergy diagnostic reagent. How to remove with chromatography like refining of common protein, A heating method (J. Allergy Clin.Immunol., 100, 171-176, 1997), although the ethyl alcohol method (Biosci.Biotechnol.Biochem, 64, 2005-2007, 2000) is known, perfect removal of an ovomucoid which affects the physical properties of a product with complicated operation is difficult -- etc., although there is a fault, When using a monoclonal antibody joint carrier, it found out that development of an ovomucoid removal method which conquered these problems was possible, and this invention was completed.

[0006]Namely, this invention is a monoclonal antibody which recognizes an ovomucoid, A monoclonal antibody to which a thermal denaturation ovomucoid does not react although it reacts to a native ovomucoid (OM1H1A, OM10D3F), Although it reacts to a thermal denaturation ovomucoid, a native ovomucoid provides a monoclonal antibody (OM6H11D) which does not react, a monoclonal antibody (OM7D3F, OM8C3C) reacted to a native ovomucoid and a thermal denaturation ovomucoid, and a hybridoma group which produces these.

[0007]According to this this invention, simple and high sensitivity ovomucoid immunoassay using an antibody which can identify a thermal denaturation state of an ovomucoid, and this antibody, and a removal method of an ovomucoid can be provided.

[0008]The identifiable monoclonal antibody can acquire a thermal denaturation state of an ovomucoid of this invention by a method already established as shown below. That is, 10-100microg of un-denaturalizing and a thermal denaturation ovomucoid is injected intraperitoneally to a mouse or a rat as suspension with a complete Freund's adjuvant etc. After performing 1 to several time booster every two to four weeks using an incomplete Freund's adjuvant, a spleen is extracted three to four days after the last immunity, the spleen cell and myeloma (myeloma) cell are united, and a hybridoma is obtained. As a myeloma cell, Y3.Ag1.2.3 etc. is used by SP-2, NS-1, P3-U1, and a rat with a mouse. A hybridoma is obtained by culturing a cell after the aforementioned cell fusion processing by the usual hybridoma selective medium (HAT medium: culture medium containing hypoxanthine, aminopterin, and thymidine).

[0009]A spleen cell cannot be increased in a HAT medium and a myeloma cell is hypoxanthine. Guanine Since it is a phosphoribosyltransferase (HGPRT) deficit stock, it cannot grow in a HAT medium containing aminopterin. Therefore, a cell which introduced a HGPRT gene from a spleen cell and has been grown in a HAT medium is a hybridoma. A hybridoma which produces the target antibody is performed with enzyme immunoassay (ELISA), limiting dilution performs single cloning after selection, and a stock stable in growth and antibody production ability is established.

[0010]Thus, the monoclonal-antibody-production hybridoma of obtained this invention can carry out subculture in the usual culture medium, and a mothball is easily possible for it in liquid nitrogen. Refining of an antibody is performed using isolation and a refining method of common protein, such as ionic exchange column chromatography, gel filtration, and affinity chromatography, using curing salting by ammonium sulfate etc., diethylaminoethylcellulose, etc.

[0011]A publicly known method indicated, for example to "enzyme immunoassay" (the 2nd edition, work besides Eiji Ishikawa, Igaku-Shoin, 1982) etc. can be used for measurement of an ovomucoid using an antibody combined with an ovomucoid of this

invention. Here, a sandwich technique based on enzyme immunoassay (ELISA) is explained briefly. Fluoroimmunoassay, chemiluminescent immunoassay, and a radioimmunity measuring method are also depended on the same principle as ELISA.
[0012]2 from which the sandwiches ELISA method differs an antigen in singularity It is the method of putting between an antibody of a seed, and after making a primary antibody (solid phase-ized antibody) and an antigen (a standard or a sample) react, a second antibody which carried out enzyme labeling to this is combined. Therefore, enzyme activity will reflect the amount of antigens inserted into two specific antibodies. The amount of antigens in a sample is computable from an analytical curve prepared using a standard isolation antigen of known concentration.
[0013]As an enzyme which carries out the sign of the antibody, they are peroxidase and beta. - Galactosidase, there are alkaline phosphatase, glucose oxidase, etc. -- these enzymes -- "enzyme immunoassay" (the biochemistry laboratory procedure 11 -- translation and) [Eiji-Ishikawa-] A sign can be carried out to an antibody by a method indicated in Tokyo Kagaku Dojin, 1989 "very-high-sensitivity enzyme immunoassay" (Eiji Ishikawa work, Japan Scientific Societies Press, 1993), etc.In order to raise sensitivity, the Avidin Biotinylated enzyme Complex (ABC) method, the Peroxidase-Anti-Peroxidase (PAP) method, etc. are used. On the other hand, as a base material of solid phase, silicon, nylon, a plastic, a bead, a microplate, or a test tube is used.
[0014]On the other hand, it is related with removal of an ovomucoid using production and this of this monoclonal antibody joint carrier which are used for ovomucoid removal, and is "Antibodies" (it and). [Harlow-&-Lane-] A publicly known method indicated to Cold Spring Harbor Laboratory, 1988, etc. can be used.
[0015]
[Example]Hereafter, although an example explains this invention still more concretely, this invention is not limited to this. 50 ml of water-soluble egg whites prepared from the preparation fresh hen's egg of the production (1) antigen of the monoclonal antibody combined with example 1 ovomucoid were mixed with 50 ml of 0.1M acetic acid buffer solution (pH 3.8). Centrifugality of the obtained egg white diluent was carried out in 4 ** after dialysis to 0.1M acetic acid buffer solution (pH 3.8) for 6000xg and 15 minutes, and NaN_3 was put in after-recovery 0.02%, and supernatant liquid was saved at 4 **, and was used as egg white liquid (a protein amount is 40mg/ml).
[0016]the 40-ml carboxymethyl group hydrophilic nature vinyl polymer gel filtration column (CM TOYOPARU 650M, TOSOH CORP. make) equilibrated with 200 ml of 0.1 M acetic acid buffer solution (pH 3.8) -- (1) 5 ml of egg white liquid and 20 ml of 0.1M acetic acid buffer solution (pH 3.8) which were obtained. Well mixed liquid was applied. The ovomucoid was eluted after washing a column with 0.1M acetic acid buffer solution (pH 3.8) with 200 ml of 0.1M acetic acid buffer solution (pH 4.3). The collected ovomucoid is 16 mg and the phosphate buffer solution (it abbreviates to the buffer solution of phosphoric acid concentration 10mM containing 150mM sodium chloride, pH 7.4, and following PBS) was presented with it as an after-dialysis native ovomucoid at the following experiments. The thermal denaturation ovomucoid was produced by heat-treating a 1mg/ml ovomucoid PBS solution by 100 ** for 1 hour.
[0017](2) They are un-denaturalizing, or ovomucoid 100 mug and the complete Freund's adjuvant () which carried out thermal denaturation as an antigen to intraperitoneal [of the female BALB/c mouse of the preparation 6-8 weeks old of immune splenocytes]. [

Freund'scomplete adjuvant and] The emulsion (1 **: 1 **) made from Difco was prescribed for the patient. 2 Medicate intraperitoneal with the emulsion (1 **: 1 **) of above-mentioned antigen 50mug and an incomplete Freund's adjuvant (Freund's imcomplete adjuvant, product made by Difco) after a week, and it is further 2. It medicated intraperitoneal with PBS containing after [a week] antigen 50mug. the 3 -- slaughtering a mouse to the Japanese backward one, extracting a spleen, and unfolding this -- a basal medium (0.3 g/l of L-glutamine made from GIBCO) They are 0.11 g/l of sodium pyruvate, 2 g/l of sodium bicarbonate, and the crystalline penicillin G potassium 1 to 25mM HEPESU content RPMI-1640 culture medium. After being suspended to the culture medium which added 10,000-unit [// l.] and streptomycin 10mg/l., spleen cells were collected by centrifugal separation.

[0018](3) Cell fusion and HAT selection (2) It is mouse myeloma cell NS-1 of the logarithmic growth phase cultivated by the prepared spleen cell and the basal medium (it is hereafter described as a serum containing medium) which added foetal calf serum 10% 5 : It mixes so that it may become a ratio of 1, and it is 2 at a basal medium. Time washing was carried out. Centrifugal separation recovers a cell, and it adds, covering 1 ml of 50% polyethylene-glycol solutions (Yamanouchi Pharmaceutical, Boehringer Mannheim) of the average molecular weight 1500 over a cell pellet for 1 minute, and is 11 after that. It settled between parts. After adding having applied a 20 more-ml basal medium for 10 minutes and diluting cell sap, centrifugal separation recovered the cell. It was suspended to a 40-ml HAT medium (serum containing medium containing 4 x10-7M aminopterin, 1.6 x10-5M thymidine, and 1 x10-4M hypoxanthine), this cell was poured distributively on four 96 hole plates, and culture was started at humidity 100 %, carbon dioxide 5 %, and 37 **. 100 mul addition of a HAT medium was done at each well, a moiety of culture media were exchanged for 2 thru/or day by day [3] with a new HAT medium after that, and culture was continued on the next day following a culture start. As a result, growth of the hybridoma was accepted by almost all wells.

[0019](4) ELISA performed screening of the hybridoma which produces the antibody combined with acquisition un-denaturalizing or the ovomucoid which carried out thermal denaturation of an antibody production hybridoma. 2 Add mug/ml of ovomucoid to 96 hole plate for ELISA every [50micro / l], and it is 1 at 37 **. After carrying out time adsorption, the plate was washed 3 times by PBS. 200 mul addition of the PBS solution (it is described as BSA-PBS below) which contains 1 % cow serum albumin in each well is done, 37 ** -- 1 -- carrying out time adsorption, and blocking each well thoroughly so that proteinic nonspecific adsorption may not start -- further -- a plate -- tris- Chloride buffer solution (it omits 10mM tris- chloride buffer solution pH7.4 containing 150mM sodium chloride and the following TBS) washed 3 times. It is the above (3) to each well. 50micro of culture supernatants l of the obtained hybridoma were added, and the antigen-antibody reaction was performed at 37 ** for 1 hour. The Tween 20 (product [made by Bio-Rad], ELISA grade) content TBS (it is hereafter described as Tween20-TBS) washed this plate once in TBS further 5 times 1 time and 0.05% in TBS, and the unreacted antibody was removed.

[0020]Next, 50microl addition of the alkaline phosphatase sign antimouse immunoglobulin G antiserum (Cappel) diluted with Tween20-TBS 1000 times to each well is done, It is 1 at 37 **. Carry out a time reaction and once by PBS in Tween20-TBS 5 times, the diethanolamine buffer solution (10% diethanolamine.) which washes once in

TBS and contains 1mg/ml of p-nitrophenyl phosphoric acid which is a substrate subsequently to each well 100 mul addition of 0.5mM $MgCl_2$ and pH 9.8 is done, It was made to react from 1 hour at a room temperature for several hours, the absorbance at 405 nm of reaction mixture was measured using the microplate leader (Model 3550, product made by Bio-Rad), and the antibody combined with the solid phase antigen was detected. As a result, one positivity well was found in five positivity wells and a thermal denaturation ovomucoid by the native ovomucoid.

[0021](5) Cloning antibody production positive well 6 of a hybridoma Cloning was performed for the cell in an individual by limiting dilution. What was added so that it might become a serum containing medium as a growth medium and might become 10% as a growth factor about ora IJIEN (solution containing the B cell growth factor made from IGEN) was used. Screening of an antibody forming cell is the above (4). By performing same ELISA and carrying out cloning of the positive clone again, 5 which produces the monoclonal antibody combined with native ovomucoid solid phase Cell strain 1H1A of a seed, 2H12D, 7D3F, 8C3C, monoclonal-antibody-production stock combined with 10D3F and thermal denaturation ovomucoid solid phase 6H11D was established.

[0022](6) About the monoclonal antibody which the determination monoclonal-antibody-production cell strain of the subclass of the immunoglobulin of a monoclonal antibody secretes in a culture supernatant fluid. The subclass of the immunoglobulin was investigated using the mouse monoclonal antibody AISO typing kit (made by Amersham Pharmacia Biotech K.K.). All monoclonal antibodies were IgG1 and the light chain was kappa.

[0023](7) In order to derive an ascites tumor to the refining BALB/c mouse of a mouse monoclonal antibody, inject 0.5 ml of pristane intraperitoneally, and it is the above (5) in the administration 3 - ten days. The monoclonal-antibody-production cell of the obtained 1×10^7 individual was transplanted to intraperitoneal. Ascites was extracted after about 2 week and the rough monoclonal antibody was obtained by saturated ammonium sulfate curing salting 50%. It purified using protein A sepharose (made by Amersham Pharmacia Biotech K.K.) further, and this rough antibody was saved by 4 ** after dialysis to PBS. About 5 mg [per ml of ascites] refining antibodies (monoclonal antibody OM1H1A, OM2H12D, OM7D3F, OM8C3C, OM10D3F, OM6H11D) were obtained.

[0024]Competition ELISA was performed for the analysis of the binding characteristic over the ovomucoid of the monoclonal antibody obtained in characteristic determination example 1 of the monoclonal antibody combined with example 2 ovomucoid. When combination with the specific antibody and solid phase-ized antigen in ELISA makes an isolation antigen live together, competition ELISA is a thing using being prevented by the capacity dependence target, and can clarify binding affinity to the antigen of an antibody according to the grade of the inhibition.

[0025](4) of Example 1 Similarly a native ovomucoid Solid-phase-izing, It is the above (7) to each blocked well. The obtained 200 ng/ml monoclonal antibody (OM1H1A, OM2H12D, OM7D3F, OM8C3C, OM10D3F, OM6H11D) 50microl, un-denaturalizing, or the thermal denaturation ovomucoid 0, ml was added every [50micro / 1] in 0.01, 0.1, 1, 10, 100, and 1000 microg /, and the antigen-antibody reaction was performed at 37 ** for 1 hour. The (4) said appearance of Example 1 was processed after that, and the alkaline-phosphatase reaction was presented with this plate. The result is shown in

drawing 1 - 3. Same competition ELISA was performed also to monoclonal antibody OM6H11D using the plate which solid-phase-ized the denaturation ovomucoid. The result is shown in drawing 4.

[0026]Since monoclonal antibody OM1H1A was strongly checked by the native ovomucoid, it became clear that the native ovomucoid was recognized specifically (drawing 1). The character as OM1H1A in which monoclonal antibody OM10D3F was the same was shown. monoclonal antibody OM2H12D -- the denaturation of isolation, and native -- neither was checked but it seemed that the canal denaturation ovomucoid accompanying solid-phase-izing is recognized specifically (drawing 2). Since comparable inhibition of monoclonal antibody OM7D3F was mostly carried out by denaturation and the native ovomucoid, it became clear that the part from which thermal denaturation is not started was recognized (drawing 3). The character as OM7D3F in which monoclonal antibody OM8C3C was also the same was shown. On the other hand, since monoclonal antibody OM6H11D was contrary to OM1H1A or OM10D3F and it was strongly prevented by the denaturation ovomucoid, it became clear that the denaturation ovomucoid was recognized specifically (drawing 4). A fixed quantity of un-denaturalizing, thermal denaturation, and the total ovomucoid is possible respectively by using the inhibition curve expressed to drawing 1 - 4 as an analytical curve.

[0027]In sensitive determination example 2 of the ovomucoid from which the thermal denaturation state by example 3 sandwiches ELISA differs. By competition ELISA using an identifiable monoclonal antibody group, it became clear about the thermal denaturation state of the ovomucoid for a fixed quantity of thermal denaturation, un-denaturalizing, and the total ovomucoid to be possible. However, since it is easy to be influenced by a coexistent substance and sensitivity is also low, competition ELISA is avidin. High sensitivity-ization by sandwiches ELISA using a biotin system was measured.

[0028]Enough biotinylation monoclonal antibody OM7D3of monoclonal antibody OM7D3F F by PBS After dialysis, It allowed to stand at the room temperature [small quantity every] to the 2 mg for 30 minutes, doing 74microl stirring of 1mg/ml Sulfo-NHS-LC-biotin (trade name :P. product made by IERCE) solution. This was enough dialyzed by PBS and it was referred to as biotinylation monoclonal antibody OM7D3F.

[0029](2) (4) Of Example 1 after solid-phase-izing 5mug/ml PBS solution 50mul of sandwiches ELISA monoclonal antibody OM10D3F, OM6H11D, and OM7D3F on an ELISA plate, respectively -- it blocked similarly. The un-denaturalizing or thermal denaturation ovomucoid 0, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000, and 10000 ng/ml Tween 20-TBS solution was added every [50micro / l] to each well, and it was neglected at 37 ** for 1 hour. A 2mug/ml biotinylation monoclonal antibody OM7D3F tween 20-TBS solution is added every [50micro / l] to each well after washing, and it is 1 at 37 **. Time neglect was carried out. A 25 ng [after Tween 20-TBS washing and also]/ml alkaline-phosphatase sign streptoavidin (OncogeneScience) Tween 20-TBS solution is added every [50micro / l], and it is 1 at 37 **. Time neglect was carried out. This plate is (4) of Example 1 after that. The alkaline-phosphatase reaction was presented similarly. The result is shown in drawing 5 - 7.

[0030]As shown in drawing 5, it is the combination of monoclonal antibody OM10D3F and biotinylation OM7D3F. In 3-300 ng/ml, only a native ovomucoid, As shown in drawing 6, only a thermal denaturation ovomucoid is almost specifically [respectively]

possible in fixed quantity in 3-100 ng/ml by monoclonal antibody OM6H11D and the combination of biotinylation OM7D3F. On the other hand, it is possible in the combination of monoclonal antibody OM7D3F and biotinylation OM7D3F in fixed quantity by the same sensitivity in a native ovomucoid and a thermal denaturation ovomucoid in 30-1000 ng/ml (drawing 7). That is, if this system is used, a fixed quantity of the total ovomucoid is possible. It has become clear that monoclonal antibody OM7D3F is a sugar chain recognition antibody of an ovomucoid. In spite of not materializing sandwiches ELISA of the monoclonal antibodies with this usually same, it enables monoclonal antibody OM7D3F to be materialized and to recognize heating and a non-heating ovomucoid equally in monoclonal antibody OM7D3F.

[0031]Pursuit of the heat denaturation process of example 4 ovomucoid (drawing 8) 3 checked in Example 3 The pursuit experiment of the heat denaturation process of an ovomucoid was conducted as an application of sandwiches ELISA of a seed. 100 It is the tube 4 with a lid every [1 / 100mu] about mug/ml of ovomucoid. It poured distributively in the book and this was quenched with ice water after 0, 10, 20, and 30-minute heating in boiling water. diluting the sample in each tube suitably, considering it as a strange sample, and performing three sorts of ELISA of Example 3 -- a native ovomucoid -- the white round head of drawing 5 -- a thermal denaturation ovomucoid -- the black dot of drawing 6 -- the total ovomucoid -- the white round head of drawing 7 -- as an analytical curve -- a fixed quantity -- the bottom.

[0032]Although many things are marketed as a carrier used for the immobilization of production protein of deovomucoid protein which used the monoclonal antibody specifically combined with example 5 ovomucoid, in this example, the example which used the affigel 10 (BIO-RAD) is given. The example using commercial ovalbumin (Sigma OVA Grade VI) is given for deovomucoid-ization as a raw material.

[0033](1) As opposed to the fixed joint buffer solution (the 0.1M sodium bicarbonate buffer solution containing 0.15M sodium chloride and pH 8.5) to resin of a monoclonal antibody. In addition to 4 ml of 50% suspension of the affigel 10 which washed 3.8mg [which was dialyzed enough]/ml monoclonal antibody OM7D3F3ml with chilled water, fall mixture is carried out at 4 ** with a room temperature overnight for 2 hours. after 3 times washing, resin, equivalent weight of 1M ethanolamine salt acid buffer solution Buffer, and pH 8.0 were added with joint buffer solution by 4 **, 500 rpm, and the centrifugality for 5 minutes, and it allowed to stand at the room temperature for 1 hour. The obtained resin was washed by PBS.

[0034](2) removal of the ovomucoid using a monoclonal antibody column -- fill in a small column fixed resin of monoclonal antibody OM7D3F produced as mentioned above, and equilibrate by PBS. In this column, 2mg/ml of dialyzed ovalbumin [1 ml of] (Sigma OVA Grade VI) was passed to PBS, and 1 ml isolated preparatively at a time at it. As a result of quantifying the ovomucoid content in the used sample and a recovery sample using the analytical curve shown in drawing 5, in the former, 0.44% of ovomucoid was mixing to ovalbumin, but in the latter, the ovomucoid was not able to be detected at all. It is proved that the ovalbumin prepared in this way also in Western analysis does not contain an ovomucoid. Although some of commercial anti-ovalbumin antibodies reacted also to the ovomucoid, the rat anti-ovalbumin antiserum produced using the ovalbumin prepared in this way did not react to the ovomucoid.

[0035]

[Effect of the Invention]In this invention, it becomes possible in fixed quantity in consideration of the thermal denaturation state about the ovomucoid which is main food allergen at minute amount high sensitivity, The results, such as allergy diagnosis, evaluation of the ovomucoid in foodstuffs and a living body, and analysis of digestion, absorption, and the migration process of foodstuffs protein, bring about a very important contribution also to basic fields, such as clinical and not only an application field like food industry but medicine, pharmaceutical sciences, agriculture, etc. This monoclonal antibody is applied also to the method of preparation of deovomucoid protein aiming at the reduction in allergen.

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* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]Drawing 1 is the competition ELISA analysis of monoclonal antibody OM1H1A.
[Drawing 2]Drawing 2 is the competition ELISA analysis of monoclonal antibody OM2H12D.
[Drawing 3]Drawing 3 is the competition ELISA analysis of monoclonal antibody OM7D3F.
[Drawing 4]Drawing 4 is the competition ELISA analysis of monoclonal antibody OM6H11D.
[Drawing 5]The fixed-quantity system of the native ovomucoid according [drawing 5] to monoclonal antibody OM10D3F and biotinylation OM7D3F
[Drawing 6]The thermal denaturation ovomucoid fixed-quantity system according [drawing 6] to monoclonal antibody OM6H11D and biotinylation OM7D3F
[Drawing 7]The fixed-quantity system of an ovomucoid which is not influenced by the thermal denaturation state according [drawing 7] to monoclonal antibody OM7D3F and biotinylation OM7D3F
[Drawing 8]Pursuit of the heat denaturation process of the drawing 8 ovomucoid

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